

## RAW SEQUENCE LISTING

**The Biotechnology Systems Branch of the Scientific and Technical  
Information Center (STIC) no errors detected.**

Application Serial Number: US/09/858,332F  
Source: IFW/6  
Date Processed by STIC: 12-2-04

# ***ENTERED***



IFW16

## RAW SEQUENCE LISTING

DATE: 12/02/2004

PATENT APPLICATION: US/09/858,332F

TIME: 11:32:13

Input Set : D:\seqlist.txt

Output Set: N:\CRF4\12022004\I858332F.raw

4 <110> APPLICANT: Tchaga, Grigory S.  
 5       Jokhadze, George  
 7 <120> TITLE OF INVENTION: Metal Ion Affinity Tags and Methods for  
 8       Using the Same  
 11 <130> FILE REFERENCE: CLON-056CIP  
 13 <140> CURRENT APPLICATION NUMBER: US 09/858,332F  
 14 <141> CURRENT FILING DATE: 2001-05-15  
 16 <150> PRIOR APPLICATION NUMBER: 09/404,017  
 17 <151> PRIOR FILING DATE: 1999-09-23  
 19 <150> PRIOR APPLICATION NUMBER: 60/101,867  
 20 <151> PRIOR FILING DATE: 1998-09-25  
 22 <160> NUMBER OF SEQ ID NOS: 27  
 24 <170> SOFTWARE: FastSEQ for Windows Version 4.0  
 26 <210> SEQ ID NO: 1  
 27 <211> LENGTH: 16  
 28 <212> TYPE: PRT  
 29 <213> ORGANISM: Artificial Sequence  
 31 <220> FEATURE:  
 32 <223> OTHER INFORMATION: affinity peptide  
 34 <400> SEQUENCE: 1  
 35 His Leu Ile His Asn Val His Lys Glu Glu His Ala His Ala His Asn  
 36   1                       5                       10                       15  
 39 <210> SEQ ID NO: 2  
 40 <211> LENGTH: 18  
 41 <212> TYPE: PRT  
 42 <213> ORGANISM: Artificial Sequence  
 44 <220> FEATURE:  
 45 <223> OTHER INFORMATION: affinity peptide  
 47 <400> SEQUENCE: 2  
 48 His Asp Asp His Asp Asp His Asp Asp His Asp Asp His Asp Asp His  
 49   1                       5                       10                       15  
 50 Asp Asp  
 54 <210> SEQ ID NO: 3  
 55 <211> LENGTH: 18  
 56 <212> TYPE: PRT  
 57 <213> ORGANISM: Artificial Sequence  
 59 <220> FEATURE:  
 60 <223> OTHER INFORMATION: affinity peptide  
 62 <400> SEQUENCE: 3  
 63 His Glu Glu His Glu Glu His Glu Glu His Glu Glu His Glu Glu His  
 64   1                       5                       10                       15  
 65 Glu Glu  
 69 <210> SEQ ID NO: 4

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70 <211> LENGTH: 18  
71 <212> TYPE: PRT  
72 <213> ORGANISM: Artificial Sequence  
74 <220> FEATURE:  
75 <223> OTHER INFORMATION: affinity peptide  
77 <400> SEQUENCE: 4  
78 His Asp Glu His Asp Glu His Glu Asn His Glu Asn His Glu Asp His  
79 1 5 10 15  
80 Glu Asp  
84 <210> SEQ ID NO: 5  
85 <211> LENGTH: 18  
86 <212> TYPE: PRT  
87 <213> ORGANISM: Artificial Sequence  
89 <220> FEATURE:  
90 <223> OTHER INFORMATION: affinity peptide  
92 <400> SEQUENCE: 5  
93 His Glu Asp His Glu Asp His Glu Asp His Glu Asp His Glu Asp His  
94 1 5 10 15  
95 Glu Asp  
99 <210> SEQ ID NO: 6  
100 <211> LENGTH: 5  
101 <212> TYPE: PRT  
102 <213> ORGANISM: Artificial Sequence  
104 <220> FEATURE:  
105 <223> OTHER INFORMATION: affinity peptide  
107 <400> SEQUENCE: 6  
108 Asp Asp Asp Asp Lys  
109 1 5  
112 <210> SEQ ID NO: 7  
113 <211> LENGTH: 4  
114 <212> TYPE: PRT  
115 <213> ORGANISM: Artificial Sequence  
117 <220> FEATURE:  
118 <223> OTHER INFORMATION: enterokinase cleavage site  
120 <400> SEQUENCE: 7  
121 Ile Glu Gly Arg  
122 1  
125 <210> SEQ ID NO: 8  
126 <211> LENGTH: 6  
127 <212> TYPE: PRT  
128 <213> ORGANISM: Artificial Sequence  
130 <220> FEATURE:  
131 <223> OTHER INFORMATION: a factor Xa cleavage site  
133 <400> SEQUENCE: 8  
134 Leu Val Pro Arg Gly Ser  
135 1 5  
138 <210> SEQ ID NO: 9  
139 <211> LENGTH: 8  
140 <212> TYPE: PRT

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141 <213> ORGANISM: Artificial Sequence
143 <220> FEATURE:
144 <223> OTHER INFORMATION: a thrombin cleavage site
146 <400> SEQUENCE: 9
147 His Pro Phe His Leu Val Ile His
148 1 5
151 <210> SEQ ID NO: 10
152 <211> LENGTH: 10
153 <212> TYPE: PRT
154 <213> ORGANISM: Artificial Sequence
156 <220> FEATURE:
157 <223> OTHER INFORMATION: a renin cleavage site
159 <400> SEQUENCE: 10
160 Cys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
161 1 5 10
164 <210> SEQ ID NO: 11
165 <211> LENGTH: 8
166 <212> TYPE: PRT
167 <213> ORGANISM: Artificial Sequence
169 <220> FEATURE:
170 <223> OTHER INFORMATION: an immunological tag
172 <400> SEQUENCE: 11
173 Asp Tyr Lys Asp Asp Asp Asp Lys
174 1 5
177 <210> SEQ ID NO: 12
178 <211> LENGTH: 11
179 <212> TYPE: PRT
180 <213> ORGANISM: Artificial Sequence
182 <220> FEATURE:
183 <223> OTHER INFORMATION: an immunological tag
185 <400> SEQUENCE: 12
186 Cys Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
187 1 5 10
190 <210> SEQ ID NO: 13
191 <211> LENGTH: 3426
192 <212> TYPE: DNA
193 <213> ORGANISM: Artificial Sequence
195 <220> FEATURE:
196 <223> OTHER INFORMATION: DNA sequence of vector containing cDNA of
197 recombinant enterokinase
199 <400> SEQUENCE: 13
200 gacgaaaggg cctcgtgata cgcctathtt tatagggttaa tgcctatgata ataatggttt 60
201 cttagacgtc aggtggcact ttctggggaa atgtgcgcgg aaccctatt tgtttathtt 120
202 tctaaatata ttcaaatatg tatccgctca tgagacaata accctgataa atgcttcaat 180
203 aatattgaaa aaggaagagt atgagtattc aacatttcctg tgcgccttt attccctttt 240
204 ttgcggcatt ttgccttctt gtttttgctc acccagaaac gctggtgaaa gtaaaagatg 300
205 ctgaagatca gttgggtgca cgagtgggtt acatcgaact ggatctcaac agcggtaaga 360
206 tccttgagag ttttcgcccc gaagaacgtt ttccaatgat gagcactttt aaagttctgc 420
207 tatgtggcgc ggtattatcc cgtattgacg cggggcaaga gcaactcggt cgccgcatac 480

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208 actatttctca gaatgacttg gttgagtact caccagtcac agaaaagcat cttacggatg 540
209 gcatgacagt aagagaatta tgcagtgtcg ccataaccat gagtgataac actgcggcca 600
210 acttacttct gacaacgatc ggaggaccga aggagctaac cgcttttttg cacaacatgg 660
211 gggatcatgt aactcgctt gatcggtggg aaccggagct gaatgaagcc ataccaaacg 720
212 acgagcgtga caccacgatg cctgtagcaa tggcaacaac gttgcgcaa ctattaactg 780
213 gcgaactact tactctagct tcccggcaac aattaataga ctggatggag gcggataaag 840
214 ttgcaggacc acttctgcgc tcggcccttc cggctggctg gtttattgct gataaatctg 900
215 gagccggtga gcgtgggtct cgcggtatca ttgcagcact ggggccagat ggtaagccct 960
216 cccgtatcgt agttatctac acgacgggga gtcaggcaac tatggatgaa cgaaatagac 1020
217 agatcgctga gatagggtgc tcaactgatta agcattggta actgtcagac caagtttact 1080
218 catatatact ttagattgat ttaaaacttc atttttaatt taaaaggatc taggtgaaga 1140
219 tcctttttga taatctcatg accaaaatcc cttaacgtga gttttcgttc cactgagggg 1200
220 cagaccccg agaaaagatc aaaggatctt cttgagatcc ttttttctg cgcgtaatct 1260
221 gctgcttgca aacaaaaaaa ccaccgctac cagcggtggt ttgtttgccg gatcaagagc 1320
222 taccaactct ttttccgaag gtaactggct tcagcagagc gcagatacca aatactgtcc 1380
223 ttctagtgtg gccgtagtta ggccaccact tcaagaactc tgtagcaccg cctacatacc 1440
224 tcgctctgct aatcctgtta ccagtggctg ctgccagtgg cgataagtcg tgtcttaccg 1500
225 ggttgactc aagacgatag ttaccggata aggcgcagcg gtcgggctga acgggggggt 1560
226 cgtgcacaca gccagcttg gagcgaacga cctacaccga actgagatac ctacagcgtg 1620
227 agctatgaga aagcggcacc cttcccgaag ggagaaaggg ggacaggtat ccggtaaagc 1680
228 gcagggtcgg aacaggagag cgcacgaggg agcttcagg gggaaacgcc tggatatctt 1740
229 atagtctgt cggttttcgc cactctgac ttgagcgtcg atttttgtga tgctcgtcag 1800
230 gggggcggag cctatggaaa aacgccagca acgcggcctt ttacgggttc ctggcctttt 1860
231 gctggccttt tgetcacatg ttctttcctg cgttatcccc tgattctgtg gataaccgta 1920
232 ttaccgcctt tgagtgaagt gataccgctc gccgcagccg aacgaccgag cgcagcgagt 1980
233 cagtgaagca ggaagcggaa gagcgcccaa tacgcaaac gcctctcccc gcgcgttggc 2040
234 cgattcatta atgcagcttg cagcagaggt ttcccagctg gaaagcgggc agtgagcgca 2100
235 acgcaattaa tgtgagttag ctcactcatt aggcacccca ggctttacac tttatgcttc 2160
236 cggctcgtat gttgtgtgga attgtgagcg gataacaatt tcacacagga aacagctatg 2220
237 accatgatta cgccaagctt gaaggatcat ctcacccaca atgtccacaa agaggagcac 2280
238 gctcatgccc acaacaagat cgatattgtc ggaggaaagt actccagaga aggagcctgg 2340
239 ccttgggtcg ttgctctgta ttctgacgat caacaggtct gcggagcttc tctggtgagc 2400
240 agggattggc tgggtgtcggc cgcccactgc gtgtacggga gaaatatgga gccgtctaag 2460
241 tggaaagcag tgctaggcct gcatatggca tcaaactgta cttctcctca gatagaaact 2520
242 aggttgattg accaaattgt cataaaccac cactacaata aacggagaaa gaacaatgac 2580
243 attgccatga tgcattctga aatgaaagtg aactacacag attatatata gcctatttgt 2640
244 ttaccagaag aaaatcaagt ttttccccca ggaagaattt gttctattgc tggctggggg 2700
245 gcacttatat atcaagggtc tactgcagac gtactgcaag aagctgacgt tccccttcta 2760
246 tcaaatgaga aatgtcaaca acagatgccg gaataataca ttacggaaaa tatggtgtgt 2820
247 gcaggctatg aagcaggagg ggtagattct tgtcaggggg attcaggcgg accactcatg 2880
248 tgccaagaaa acaacagatg gctcctggct ggcgtgacgt catttgata tcaatgtgca 2940
249 ctgcctaata gccacggggg gtatgcccg gtcceaagg tccacagagt gatacaaagt 3000
250 tttctacatg agctcgtaat tagctgagaa ttcactggcc gtcgttttac aacgtcgtga 3060
251 ctgggaaaaa cctggcggtt cccaacttaa tcgccttgca gcacatcccc ctttcgccag 3120
252 ctggcgtaat agcgaagagg cccgcaccga tcgccttcc caacagttgc gcagcctgaa 3180
253 tggcgaatgg cgcctgatgc ggtattttct ccttacgcat ctgtgcggta tttcacaccg 3240
254 catatggtgc actctcagta caatctgctc tgatgccgca tagttaagcc agccccgaca 3300
255 cccgccaaac cccgctgacg cgcctgacg ggcttgctct ctcgccgcat ccgcttacag 3360
256 acaagctgtg accgtctccg ggagctgcat gtgtcagagg ttttcaccgt catcaccgaa 3420

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Input Set : D:\seqlist.txt

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```

257 acgcgc
259 <210> SEQ ID NO: 14
260 <211> LENGTH: 269
261 <212> TYPE: PRT
262 <213> ORGANISM: Artificial Sequence
264 <220> FEATURE:
265 <223> OTHER INFORMATION: protein sequence of vector containing cDNA of
266 recombinant enterokinase
268 <400> SEQUENCE: 14
269 Met Thr Met Ile Thr Pro Ser Leu Lys Asp His Leu Ile His Asn Val
270 1 5 10 15
271 His Lys Glu Glu His Ala His Ala His Asn Lys Ile Asp Ile Val Gly
272 20 25 30
273 Gly Ser Asp Ser Arg Glu Gly Ala Trp Pro Trp Val Val Ala Leu Tyr
274 35 40 45
275 Phe Asp Asp Gln Gln Val Cys Gly Ala Ser Leu Val Ser Arg Asp Trp
276 50 55 60
277 Leu Val Ser Ala Ala His Cys Val Tyr Gly Arg Asn Met Glu Pro Ser
278 65 70 75 80
279 Lys Trp Lys Ala Val Leu Gly Leu His Met Ala Ser Asn Leu Thr Ser
280 85 90 95
281 Pro Gln Ile Glu Thr Arg Leu Ile Asp Gln Ile Val Ile Asn Pro His
282 100 105 110
283 Tyr Asn Lys Arg Arg Lys Asn Asn Asp Ile Ala Met Met His Leu Glu
284 115 120 125
285 Met Lys Val Asn Tyr Thr Asp Tyr Ile Gln Pro Ile Cys Leu Pro Glu
286 130 135 140
287 Glu Asn Gln Val Phe Pro Pro Gly Arg Ile Cys Ser Ile Ala Gly Trp
288 145 150 155 160
289 Gly Ala Leu Ile Tyr Gln Gly Ser Thr Ala Asp Val Leu Gln Glu Ala
290 165 170 175
291 Asp Val Pro Leu Leu Ser Asn Glu Lys Cys Gln Gln Gln Met Pro Glu
292 180 185 190
293 Tyr Asn Ile Thr Glu Asn Met Val Cys Ala Gly Tyr Glu Ala Gly Gly
294 195 200 205
295 Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Cys Gln Glu
296 210 215 220
297 Asn Asn Arg Trp Leu Leu Ala Gly Val Thr Ser Phe Gly Tyr Gln Cys
298 225 230 235 240
299 Ala Leu Pro Asn Arg Pro Gly Val Tyr Ala Arg Val Pro Arg Phe Thr
300 245 250 255
301 Glu Trp Ile Gln Ser Phe Leu His Glu Leu Val Ile Ser
302 260 265
305 <210> SEQ ID NO: 15
306 <211> LENGTH: 12
307 <212> TYPE: PRT
308 <213> ORGANISM: Artificial Sequence
310 <220> FEATURE:
311 <223> OTHER INFORMATION: an amino acid sequence embodiment of the affinity

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RAW SEQUENCE LISTING ERROR SUMMARY  
PATENT APPLICATION: US/09/858,332F

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Input Set : D:\seqlist.txt

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Please Note:

Use of n and/or Xaa have been detected in the Sequence Listing. Please review the Sequence Listing to ensure that a corresponding explanation is presented in the <220> to <223> fields of each sequence which presents at least one n or Xaa.

Seq#:21; Xaa Pos. 2,3,5,6,7,9

Seq#:23; Xaa Pos. 2,3,5,6,8,9

**VERIFICATION SUMMARY**

DATE: 12/02/2004

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TIME: 11:32:14

Input Set : D:\seqlist.txt

Output Set: N:\CRF4\12022004\I858332F.raw

L:395 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:21  
L:400 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:21  
L:405 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:21  
L:410 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:21  
L:415 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:21  
L:416 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:21 after pos.:0  
L:446 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:23  
L:450 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:23  
L:454 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:23  
L:458 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:23  
L:462 M:258 W: Mandatory Feature missing, <220> Tag not found for SEQ ID#:23  
L:463 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:23 after pos.:0